Review article

The potential of CRISPR-Cas9 for treating genetic disorders

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The CRISPR (clustered, regularly interspaced, short palindromic repeats)-Cas9 (CRISPR-associated protein 9) system is a targeted nuclease technology that allows precise genome editing. Since the system was first demonstrated for use in genome editing, there has been huge interest generated in evaluating its potential for human gene therapy, and it has most recently been used to modify human embryos for the purpose of gene therapy. The CRISPR-Cas9 system has multiple advantages in comparison to the pre-existing targeted nucleases transcription activator-like effector nucleases and zinc finger nucleases, which are discussed here. However, as a relatively new genome editing platform, safety issues such as off-target editing have yet to be fully investigated. In order to develop CRISPR-Cas9-based gene therapy, diseases amenable for targeting must first be selected, alongside appropriate and efficient delivery methods. This review addresses these challenges and current strategies for improvement, as well as the inherent socio-ethical considerations that surround the use of human genome editing.

Key words: CRISPR-Cas9, gene therapy, genome editing, biotechnology, endonuclease, off-target effects

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Introduction

The appeal of gene therapy lies in its potential to permanently repair the disease-causing sequence, thus preventing the need for further treatment. One early major challenge within gene therapy was increasing the rate at which homologous recombination (HR)-mediated gene targeting occurs in human cells, so that the desired product could be inserted into the genome (Zwaka and Thomson, 2003). Programmable nucleases, which allow precise modification of the target locus, have been developed in recent years to address this problem. These recognize and bind a target DNA sequence and subsequently induce the formation of a double-stranded break (DSB) in the target region, which following repair can significantly increase the rate of HR (Mali et al., 2013).

DSBs are repaired using one of two endogenous repair pathways in the cell; non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Fig. 1B). The NHEJ pathway occurs throughout the cell cycle, repairing DSBs through end-to-end joining of DNA strands (Deriano and Roth, 2013). HDR occurs with much lower frequency during only the replicative phase of the cell cycle, repairing breaks using the homologous sister chromatid as a template (Mao et al., 2008; Vartak and Raghavan, 2015). In the context of gene editing, NHEJ is error-prone and therefore useful for introducing random mutations to target DNA, whereas HDR is preferable when a donor DNA template is to be inserted into the genome (Hsu, Lander and Zhang, 2014). As both NHEJ and HDR occur concurrently in the cell, the efficiency of HDR depends on the frequency at which NHEJ occurs (Maruyama et al., 2015). Inhibitors of DNA ligase IV, a key enzyme in NHEJ, have recently been demonstrated to significantly enhance HDR frequency, and may therefore be co-delivered with programmable nucleases in order to improve the efficiency of HDR-mediated insertional mutagenesis (Chu et al., 2015; Maruyama et al., 2015).
The recently developed CRISPR (clustered, regularly interspaced, short palindromic repeats)-Cas9 (CRISPR-associated protein 9) system is unique among engineered nucleases in that its specificity is conferred by Watson–Crick base pairing (bp) between a sgRNA molecule and the target DNA (Jinek et al., 2012) (Fig. 1A). This property makes it easily customizable and relatively simple to use; appealing attributes for gene therapy (Ran et al., 2013). The technology originated from a bacterial form of RNA-mediated adaptive immunity, demonstrated to cleave foreign DNA (Bolotin et al., 2005). The mechanism is the prokaryotic equivalent of eukaryotic RNA-interference, in which RNA transcripts are used to guide endonucleases to destroy invading viral or plasmid DNA (Marraffini and Sontheimer, 2010).

The Type II CRISPR-Cas9 mechanism originating from Streptococcus pyogenes has been harnessed for genome editing by using the Cas9 protein directed by a 20nt sequence in a sgRNA (Fig. 1A) (Mali et al., 2013). CRISPR-Cas9-mediated genome editing has been performed in a wide range of human cell lines including human embryonic kidney cells (Mali et al., 2013) induced pluripotent stem cells (iPSCs) (Smith et al., 2011), and most recently in early human embryos (Liang et al., 2015). The potential for CRISPR-Cas9 in clinical applications is promising and has prompted the emergence of multiple pharmaceutical start-ups developing CRISPR-Cas9-mediated gene therapy (Shen, 2013).

**Comparison with other genome editing platforms**

Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were engineered to recognize and subsequently cleave target DNA through protein–DNA interactions. Both require dimerization of the FokI domain to induce DSBs (Christian et al., 2010; Pattanayak et al., 2011). CRISPR-Cas9 technology differs from ZFNs and TALENs as it is derived from a naturally occurring mechanism and is targeted by bp between the sgRNA and target DNA (Jinek et al., 2012).

ZFNs are a well-established editing platform, and they are presently being used in clinical trials for treating HIV (Tebas et al., 2014). However, they are difficult to engineer with the required targeting efficiency and specificity for therapeutic applications as they require labour-intensive protein engineering (Urnov et al., 2010). In contrast to ZFNs, which recognize DNA triplets, TALENs recognize single bp, allowing greater design flexibility (Boch, 2011). TALENs are simpler to design than ZFNs, but still require complex molecular cloning methods (Miller et al., 2011). CRISPR-Cas9 technology is comparatively easy to design as it only requires changing the sgRNA sequence to target the desired locus, and thus can be engineered using standard
molecular biology cloning and synthesis procedures (Mali et al., 2013; Table 1).

One constraint of CRISPR-Cas9 technology is the PAM requirement for the target DNA, which must be found 3 bp upstream of the target sequence. The system derived from S. pyogenes requires the PAM 5′-NGG-3′, which occurs on average every 8–12 bp within the human genome (Cong et al., 2013; Hsu, Lander and Zhang, 2014). As this is an average, it cannot be guaranteed that a PAM will be found adjacent to the target sequence. Directed evolution or use of Cas9 from other bacteria may be a solution to this constraint by enabling use of alternate PAM sequences or PAM-independence (Esvelt et al., 2013).

Due to its relatively recent discovery, the mechanism underlying CRISPR-Cas9-mediated editing is not yet as well understood and will therefore require further study before clinical use. Increased study of the DNA recombination and repair machinery of the cell could also help to increase efficiency of targeting (Sung and Klein, 2006). The ease of customization of CRISPR-Cas9 combined with its comparable rates of targeting efficiency makes it appealing for gene therapy as it could be appropriately modified for different individuals (Sung et al., 2014).

**Potential disease targets**

Genetic diseases most amenable for CRISPR-Cas9 editing are those in which a single allele needs to be targeted, as biallelic targeting has much lower efficiency (Ye et al., 2014). Complex diseases may yet be too difficult to treat using CRISPR-Cas9, because multiple mutations are often involved which may be difficult to target simultaneously. However, the unique multiplexing ability of CRISPR-Cas9 may make simultaneous targeting a possibility in the near future (Cong et al., 2013). Diseases targeted so far using CRISPR-Cas9 are summarized in Table 2. For diseases that result from the production of pathogenic gene products, CRISPR-Cas9 can be used to disrupt the dominant allele by NHEJ. If the disease is caused by the loss-of-function of a gene, it can be corrected using the HDR pathway by providing a functioning copy of the gene on a donor template (Fig. 1B) (Ran et al., 2013).

Gene therapy is ideal where cells gain a selective advantage when the causative mutation is repaired (Neff, Beard and Kiem, 2006). This was demonstrated successfully by using CRISPR-Cas9 to correct a mutation in FAH in adult mice; a model of human hereditary tyrosinemia type I. In this study, only 0.4% of hepatocytes were initially repaired, yet the strong positive selection of Fah+ cells confers substantial therapeutic effect (Yin et al., 2014). Diseases that can be treated using stem cell transplantation can effectively increase the targeting efficiency to 100% through selection of edited cells. Many proof-of-concept studies have shown this to be a feasible method for correcting genetic diseases (Table 2). X-linked severe combined immunodeficiency (X-SCID) and sickle-cell anaemia can be treated by autologous transplantation of edited hematopoietic stem cells. Subsequent in vivo selection and proliferation is then sufficient to restore normal function (Neff, Beard and Kiem, 2006; Xie et al., 2014b). However, for diseases such as cystic fibrosis where transplantation of stem cells is not an option, in vitro editing is required. Here it is essential that cells can be targeted at a sufficient rate by viral vectors to achieve therapeutic benefit (Schwank et al., 2013).

CRISPR-Cas9 can be used in antiviral strategies, by introducing viral resistance genes or targeting proviral DNA. Individuals who have CCR5Δ32 genes are resistant to HIV-1 infection (Sheppard et al., 2002). A recent study utilized CRISPR-Cas9 to induce this mutation in human iPSCs and thus enabled resistance to HIV-1 infection (Ye et al., 2014). CRISPR-Cas9 has also been used to cleave and inactivate the genomes of Epstein-Barr virus and HIV-1 in infected human cell lines (Yuen et al., 2015; Zhu et al., 2015). These are promising results, and combined with the use of iPSCs may provide a safer transplant-based method of treating HIV-1 infection (Tebas et al., 2014).

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**Table 1.** Comparison of three engineered nucleases presently used for gene editing: ZFNs, TALENs and CRISPR-Cas9

<table>
<thead>
<tr>
<th></th>
<th>ZFNs</th>
<th>TALENs</th>
<th>CRISPR-Cas9</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanism</td>
<td>Protein-guided DNA endonuclease</td>
<td>Protein-guided DNA endonuclease</td>
<td>RNA-guided DNA endonuclease</td>
<td>Hsu, Lander and Zhang (2014)</td>
</tr>
<tr>
<td>Efficiency</td>
<td>Low (~10%)</td>
<td>High (~20%)</td>
<td>High (~20%)</td>
<td>Kim and Kim (2014)</td>
</tr>
<tr>
<td>Off-target effects</td>
<td>High</td>
<td>Low</td>
<td>Varies with target sequence</td>
<td>Pattanayak et al. (2011), Wang et al. (2015)</td>
</tr>
<tr>
<td>Design</td>
<td>Difficult</td>
<td>Moderate</td>
<td>Easy</td>
<td>Urnov et al. (2010), Miller et al. (2011), Mali et al. (2013)</td>
</tr>
<tr>
<td>Requirements</td>
<td>G-rich sequences</td>
<td>5′T and 3′A</td>
<td>PAM</td>
<td>Hsu, Lander and Zhang (2014)</td>
</tr>
</tbody>
</table>
Table 2. Summary of genetic diseases that have been successfully corrected in cells using CRISPR-Cas9 technology

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mutation target</th>
<th>Delivery method</th>
<th>Target cells</th>
<th>Correction efficiency</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Thalassaemia</td>
<td>Deletion in HBB</td>
<td>Electroporation</td>
<td>Human iPSCs</td>
<td>17.6%</td>
<td>Xie et al. (2014b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intracytoplasmic injection</td>
<td>Human tripolarzygotes</td>
<td>14.3%</td>
<td>Liang et al. (2015)</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>Deletion in CFTR</td>
<td>Lipofection</td>
<td>Human intestinal organoids</td>
<td>Unknown-edited cells were selected</td>
<td>Schwank et al. (2013)</td>
</tr>
<tr>
<td>Hereditary tyrosinemia</td>
<td>Point mutation in FAH</td>
<td>Hydrodynamic injection</td>
<td>in vivo mice hepatocytes</td>
<td>0.40 ± 0.12%</td>
<td>Yin et al. (2014)</td>
</tr>
<tr>
<td>HIV-1</td>
<td>CCRSΔ32</td>
<td>Electroporation</td>
<td>Human iPSCs</td>
<td>100%</td>
<td>Zhu et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleofection</td>
<td>JLat10.6 cells</td>
<td>30%</td>
<td>Ye et al. (2014)</td>
</tr>
<tr>
<td>Duchenne muscular dystrophy</td>
<td>Exon deletion in dystrophin gene</td>
<td>Electroporation</td>
<td>Human iPSCs</td>
<td>50%</td>
<td>Li et al. (2015)</td>
</tr>
<tr>
<td>α1-Antitrypsin deficiency</td>
<td>Point mutation in SERPINA1</td>
<td>Electroporation</td>
<td>Human iPSCs</td>
<td>18.8%</td>
<td>Smith et al. (2014a)</td>
</tr>
<tr>
<td>Polycythaemia vera</td>
<td>Point mutation in JAK2</td>
<td>Electroporation</td>
<td>Human iPSCs</td>
<td>9.15%</td>
<td>Smith et al. (2014a)</td>
</tr>
<tr>
<td>Cataracts</td>
<td>Deletion in Crygc</td>
<td>Electroporation</td>
<td>Mouse spermatogonial stem cells</td>
<td>29.7%</td>
<td>Wu et al. (2014)</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>Inactivation of viral promoter</td>
<td>Electroporation</td>
<td>Human epithelial cell lines</td>
<td>94.2%</td>
<td>Yuen et al. (2015)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Disruption of Pcsk9</td>
<td>Adenovirus</td>
<td>in vivo mice hepatocytes</td>
<td>50%</td>
<td>Ran et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adeno-associated virus</td>
<td>Human epithelial cell lines</td>
<td>40%</td>
<td>Ding et al. (2014)</td>
</tr>
</tbody>
</table>

CCRSΔ32, 32 bp deletion within C-C chemokine receptor type 5; CFTR, cystic fibrosis transmembrane conductance regulator; Crygc; crystallin gamma c; FAH, fumarylacetoacetate hydrolase; HBB, human haemoglobin beta; HIV-1, human immunodeficiency virus type 1; JAK2, janus kinase 2; LDL-C, low-density lipoprotein cholesterol; Pcsk9, proprotein convertase subtilisin/kexin type 5; SERPINA1, serpin peptidase inhibitor; clade A (alpha-1 antiproteinase, antitrypsin); member 1.

**Safety concerns: off-target effects**

One major concern with the therapeutic use of engineered nucleases is their potential for off-target mutagenesis. A previous gene therapy trial for X-SCID conducted in 2001 led to the development of leukaemia in patients due to insertional oncogenesis of the viral vector (Kohn, Sadelain and Glorioso, 2003). There is thus a need to thoroughly assess the risk of CRISPR-Cas9 inducing harmful changes to the target genome. Recent studies have reported evidence of CRISPR-Cas9 inducing off-target mutations (Fu et al., 2013; Hsu et al., 2013; Wang et al., 2015). Cas9 can tolerate up to five mismatches within the 23nt sgRNA sequence, which may result in unwanted chromosomal rearrangements such as insertions, deletions or translocations (Cho et al., 2014). Levels of off-target effects (OTEs) depend on factors such as guanine-cytosine content (GC) content and chromatin accessibility (Ren et al., 2014). The latter is a concern as it may confer increased levels of OTEs within expressing genes (Wu et al., 2014). Potential off-target sites can be determined computationally, as specificity is directly related to the number of mismatches between the sgRNA and target DNA (Hsu et al., 2013). These sites can then be analysed using strategies developed for detecting Cas9-induced mutations.

One strategy is to use mismatch-specific endonucleases such as T7E1 and Surveyor that are able to detect heteroduplex DNA that forms as a result of mismatches (Cong et al., 2013). This is relatively cheap and simple to use, allowing initial screening for OTEs in one day (Vouillot, Thelie and Pollet, 2015). However, they have relatively low sensitivities, and this technique is also biased towards bio-informatically...
predicted sites; in silico effects do not always match those observed in vivo (Wang et al., 2015). Several studies do not use unbiased methods for the detection of OTEs, and thus reported an apparent absence of off-targeting, yet do not account for the possibility of undetected mutations (Friedland et al., 2013; Ding et al., 2014; Smith et al., 2014a; Xie et al., 2014b).

Alternatives such as whole genome sequencing offer an unbiased and more comprehensive approach. Deep sequencing of an edited genome is more sensitive than the use of T7E1 or Surveyor assays, and can detect mutations that occur at frequencies from 0.01% to 0.1% (Smith et al., 2014b). Digenerate-seq is a recently developed method that sequences in vitro Cas9-digested genomes from a population of edited cells, allowing detection of rare off-target mutations (Kim et al., 2015) which would be useful for Cas9-mediated stem cell therapy.

In some instances, Cas9 treatment does not cause significantly higher levels of mutations that occur in non-transfected control cells (Koike-Yusa et al., 2014). With the case of severe genetic diseases such as sickle-cell anaemia, if this approach offers better prospects than other existing chemical therapies, it may be that the potential benefits outweigh the risk of OTEs. Nevertheless, as specificities of different sgRNAs can widely vary (Kim et al., 2015), their potential for OTEs should be thoroughly assessed using multiple methods before considering clinical application.

**Strategies for improving safety**

A number of bioinformatic resources have been developed for the design of more specific sgRNAs that minimize OTEs, and can also be used for detecting potential off-target sites. These allow highly specific and active sgRNAs to be designed quickly and cheaply (Montague et al., 2014; Xie et al., 2014a; Prykhozhij et al., 2015). Changing various parameters of sgRNA design can also reduce the levels of OTEs. The use of sgRNAs with two additional guanine nucleotides at the 5' end and truncated sgRNAs with shorter complementary regions increases specificity, although this may be at the sacrifice of on-target efficiency (Cho et al., 2014; Fu et al., 2014). Lowering the GC content of sgRNAs can also achieve higher specificity (Ren et al., 2014); however, the ability to do this will depend on the target locus.

Modifications have been made to the Cas9 protein in order to optimize genome editing and minimize OTEs. The Cas9 nickase mutant (Cas9n) has been developed with one inactivated catalytic domain. Thus, instead of inducing a DSB, it creates a single-stranded break or ‘nick’ in the target region. By using paired Cas9 nickases that generate two single-stranded breaks on opposite strands, this method doubles the target recognition sequence of Cas9 (Ran et al., 2013) (Fig. 2A). This effectively doubles the specificity of editing as it requires two independent binding and cleavage events. Using paired nickases has also been shown to increase efficiency of HDR, and so would be useful when inserting a corrected allele (Cho et al., 2014).

A self-destruct mechanism has been developed that allows temporal control of Cas9 expression. The system can be finely-tuned to adjust expression duration and amplitude. It is composed of a self-cleaving vector that can be delivered to cells (Moore et al., 2015) (Fig. 2B). Constitutive expression of Cas9 in cells could be potentially toxic, and would need to be precisely controlled for gene therapy (Jiang et al., 2014). This method would enable control of delivery dosage as well as ensuring that Cas9 is only expressed for a safe length of time. Cas9 activity can also be spatially controlled, through the use of light-inducible heterodimeric domains. This technology is derived from the CRY2 and CIB1 proteins of Arabidopsis thaliana, and allows activation of Cas9 after exposure to blue light (Polstein and Gersbach, 2015) (Fig. 2C). Small molecules could also potentially be similarly used to induce activity (Hsu, Lander and Zhang, 2014). This could prove useful when expression needs to be targeted to specific organs within a patient, such as with cystic fibrosis.

The piggyBac transposase tool allows ‘seamless’ editing, in which no trace of the donor vector is left within the target genome (Li et al., 2013) (Fig. 3). Its use has been demonstrated in conjunction with Cas9 for seamless gene correction in human iPSCs, and presents a safe solution to ensuring no sequences with unpredictable effects are inserted into the target region (Ye et al., 2014; Xie et al., 2014b; Li et al., 2015). For future therapeutic applications, it may be appropriate to first assess the level of off-target mutagenesis induced by the selected sgRNA, and then make modifications to the editing procedure if required. An appropriate balance between specificity and efficiency will need to be found.

**Delivery methods: in vivo vs. ex vivo**

Delivery is a major hurdle that needs to be overcome before proof-of-concept studies can be translated into a clinical setting. It is not plausible to correct the disease mutation in all cells of an individual; instead, approaches aim to treat a sufficient number of cells in the appropriate location in order for a therapeutic benefit to be seen. Three components are delivered to cells; the Cas9 protein, the sgRNA and a repair template carrying the corrected allele, if required (Mali et al., 2013). This can be delivered in a single ‘all-in-one’ DNA vector or as Cas9 protein with the sgRNA. Protein delivery has the advantage of allowing precise dosage control (Kim et al., 2014), whereas vector delivery provides a simple method of selection for edited cells (Mali et al., 2013). In recent studies, transfection of vectors via electroporation is the most common delivery method used (Table 2).

As previously discussed, diseases most amenable for gene therapy are those which can be targeted ex vivo. Gene editing technology can be combined with the use of iPSCs, where patient-specific iPSCs are generated, edited ex vivo, and then transplanted back into the individual (Xie et al., 2014b; Li...
et al., 2015). This method has minimal OTEs (Suzuki et al., 2014). It provides the possibility of ‘personalized cell therapy’, in which there is little risk of immune rejection by the individual receiving the therapy (Li et al., 2014). However, there are multiple issues associated with stem cell transplantation, such as poor cell survival and engraftment (Harding et al., 2015).

Figure 2. Modifications of CRISPR-Cas9 technology for improving safety in gene therapy. (A) Two Cas9n complexes with paired sgRNAs mediate simultaneous single-stranded breaks in the target gene. (B) CRISPR-based self-cleaving mechanism for control of Cas9 expression. The sgRNA-Cas9 complex cleaves both target DNA and within the delivery vector, resulting in self-degradation. (C) Split heterodimeric domains of Cas9 fused to CRY2 and CIB1 proteins dimerize upon exposure to blue light, allowing spatial control of activity. (A,C) Adapted from Cell, 157 (6), Hsu et al., copyright (2014), with permission from Elsevier. (B) Adapted from Moore et al., copyright (2015), with permission from Oxford University Press.

Figure 3. Seamless correction of a genetic disorder using the piggyBac transposase tool and CRISPR-Cas9. Cas9 creates a DSB within the target gene, which mediates insertion of the piggyBac construct via HDR. The piggyBac construct carries a functioning copy of the target gene, selectable markers in order to select for edited cells and is flanked by inverted terminal repeats and wild-type sequences in order to mediate HDR. Expression of piggyBac transposase triggers excision of the transposon, leaving only the corrected gene within the target genome. Adapted from Sun and Zhao, copyright (2014), with permission from John Wiley and Sons.
Current transplantation technology may, therefore, need to improve before CRISPR-Cas9 can be used in a clinical setting.

For diseases where stem cell transplantation is not an option, in vivo delivery of the components is required. The most effective method will be to use viral delivery vectors carrying the required components, compensating for the disadvantages of either; viral vectors may integrate randomly yet Cas9 edits specifically. The viral vectors allow higher efficiency targeted editing (Suzuki et al., 2014). Helper-dependent adenoviral vectors that have been developed for gene therapy are ideal for this approach as they are able to transduce both dividing and non-dividing cells, have low immunogenicity and are episomal so there is a reduced risk of viral integration (Maggio et al., 2014). Their large cloning capacity of 36 kb allows for delivery of the full length Cas9 protein alongside other required components (Gonçalves and de Vries, 2006). Adenoviral delivery of CRISPR-Cas9 has been used to successfully disrupt the mouse Pcsk9 gene in vivo in order to reduce blood levels of LDL-C (Ding et al., 2014). This system is effective in a wide range of human cells, with targeting efficiencies of up to 65% (Holkers et al., 2014; Maggio et al., 2014).

One challenge of using viral vectors for gene therapy is the immune response that can be generated (Ding et al., 2014). Use of adeno-associated virus (AAV) vectors would therefore be preferable as they generally have lower levels of immunogenicity than HDAdVs, and reduced risk of insertional onco- genesis (Vasileva and Jessberger, 2005). Unfortunately, the 4140 bp size of Cas9 exceeds the 2.4 kb packaging capacity of AAV vectors (Senis et al., 2014). The use of smaller Cas9 orthologs derived from other bacteria allows the system to be delivered using AAV vectors (Esvelt et al., 2013). For example, Cas9 derived from Staphylococcus aureus is 1 kb shorter than that of S. pyogenes, and has been delivered using an AAV vector to target Pcsk9 in mice (Ran et al., 2015). This method had lower immunogenicity than the study that used an adenovirus vector, but was 10% less efficient (Table 2) (Ding et al., 2014; Ran et al., 2015). As with OTEs, an appropriate balance between safety and efficiency must be met for future therapeutic use.

Conclusions

There are various ethical issues with the application of genome engineering, and they are often closely linked with the associated safety concerns. Due to the rapid speed with which CRISPR-Cas9 use has progressed, groups of scientists within the field recently called for a moratorium on performing human germline editing (Baltimore et al., 2015; Lannphier et al., 2015). There is concern over the unpredictable effects the technology could have if used in embryos; harmful mutations may be accidentally introduced and passed on to future generations. Another concern is that CRISPR-Cas9 technology may be used for non-therapeutic applications, enhancing desirable factors in a move towards ‘designer babies’ (Vogel, 2015). There are potential benefits of germline editing, such as preventing future inheritance of genetic diseases, yet without first improving safety of editing within somatic cells, germline editing is unlikely to be used therapeutically in the foreseeable future (Cyranoski and Reardon, 2015).

Despite the calls for a moratorium, a recent study demonstrated the first example of human germline editing, in which CRISPR-Cas9 was used to target the HBB gene within human embryos. High levels of OTEs were observed, and the authors concluded that further understanding of the mechanism of CRISPR-Cas9-mediated genome editing is essential (Liang et al., 2015). Scientists have widely condemned the study, as the genetic modification of human embryos is likely to draw widespread public criticism due its attendant socio-ethical implications. This could in turn impede important progress towards the therapeutic use of CRISPR-Cas9.

Overall, there is still much progress that needs to be made to ensure the safety of CRISPR-Cas9 in therapy. OTEs are a major concern and the system cannot proceed to clinical trials before these are fully addressed. Similarly, in vivo delivery methods will need to be improved in order to provide sufficient benefit for diseases that cannot be targeted ex vivo. The majority of studies using CRISPR-Cas9 for gene editing published thus far are still ‘proof-of-concept’, but multiple start-up pharmaceutical companies such as Editas Medicine are now aiming to use CRISPR-Cas9 technology to develop treatments (Shen, 2013). These will hopefully pave the way to the future use of CRISPR-Cas9 as a safe and effective form of treatment for a variety of genetic disorders.

Author biography

I am a student at Imperial College London, where I study Biology (Bsc) with a Year in Industry. This year I am undertaking an industrial placement at GlaxoSmithKline, where I am working in the Allergic Inflammation Discovery Performance Unit within their Respiratory Therapeutic Unit. The placement has already allowed me to gain a large amount of laboratory experience and an insight into the pharmaceutical industry. I very much look forward to increasing my skill set in the year ahead. After completing my degree, I wish to continue in my studies by pursuing a PhD, with a particular focus on the fields of immunology and gene therapy. Ultimately, I wish to have a career in research, as it would allow me to directly contribute to advancing biological science and developing treatments for life threatening diseases.

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